Exhibit A

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Boyle, William J.

Serial No.: 09/211,315 Group Art Unit No.: 1644

Filed: December 14, 1998 Examiner: Turner, S.

For: Osteoprotegerin Binding Proteins

Docket No.: A-451G

DECLARATION OF JOHN K. SULLIVAN

Assistant Commissioner for Patents
Box AF
Washington, D.C. 20231

Sir:

I, John K. Sullivan, declare and state that:

1. I am presently employed by Amgen Inc. as an Associate Scientist in the Department of Inflammation and have held this position since May of 1991. My research interests are in the area of rheumatology and immunobiology.

2. I received my B.S. from Michigan Technological Institute in 1982 and a M.S. in Biology from Wright State University in 1984.

3. I have been asked to provide evidence concerning whether, based on the disclosure of U.S. Serial No. 09/211,315 (hereafter the '315 application), one of skill in the art without undue experimentation would have been able to obtain antibodies which bind osteoprotegerin binding protein (OPGbp) and modulate

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231, on the date appearing below.

August 18, 2000
Date

Sherry St. Andrew by Lynne Buchsbaum
Signature

the activity of OPGbp such bone resorption is inhibited. The evidence presented in this declaration shows that the teachings of the '315 application would have enabled one to obtain antibodies which are suitable for use in the methods claimed in the '315 application. My conclusion is that the '315 application enables one skilled in the art to carry out the claimed methods without undue experimentation.

4. In arriving at this conclusion, I have read and understood the work of Dr. William J. Boyle in the '315 application, including Example 3 which describes the sequencing of DNA encoding murine OPGbp, Example 5 which describes the cloning and sequencing of DNA encoding human OPGbp, Examples 8 and 9 which describe *in vitro* and *in vivo* assays for OPGbp activity, and Example 11 which describes materials and procedures for the preparation of anti-OPGbp antibodies. Based on the work of Dr. Boyle in the '315 application, work done under my direction and control as set forth herein, and work done by others which is set forth herein based upon information provided to me, I conclude that the '315 application enables one skilled in the art to carry out the claimed invention without undue experimentation.

5. The following OPGbp peptides and polypeptides were used in the experiments described herein:

A BB' loop-Cys peptide having the amino acid sequence set forth on p. 47, line 18 of the specification and in SEQ ID NO:33 (hereafter "BB' loop-Cys").

A EF loop6-Cys peptide having the amino acid sequence as follows:

KTSIKIPSSHNLMKC

(hereafter "EF loop6-Cys").

Murine OPGbp[158-316] having the amino acid sequence from positions 158 to 316 inclusive as set forth in SEQ ID NO:37 of the '315 application and expressed and purified generally as described in Examples 6 and 7, respectively, of the '315 application.

Human OPGbp[159-317] having the sequence from positions 159 to 317 inclusive as set forth in SEQ ID NO:39 of the '315 application and expressed and purified generally as described in Examples 6 and 7, respectively, of the '315 application.

Based on information provided to me, the BB' loop-Cys and EF loop6-Cys peptides were conjugated with keyhole limpet hemocyanin (KLH) prior to immunization.

6. Human OPGbp[159-317] and the KLH-conjugated BB' loop-Cys and EF loop6-Cys peptides were provided to a contract laboratory for immunization of New Zealand white rabbits. Based on information provided to me, serum antibody titers of immunized rabbits were determined by EIA generally as described on p. 48, line 28 to p. 50, line 5 using either human OPGbp[159-317], BB' loop-Cys or EF loop6-Cys peptide coated on microtiter plates.

7. Crude antisera from immunized rabbits were purified by affinity chromatography. Antisera from rabbits immunized with human OPGbp[159-317] were provided to the Genomics Department at Amgen Inc. and purified by applying to an Actigel Ald column coupled to human OPGbp[159-317] and eluting with Pierce Gentle Elution Buffer (Pierce) containing 1% glacial acetic acid. Antisera from rabbits immunized with BB' loop-Cys peptide were provided to the Protein Chemistry Department at Amgen Inc. and purified by applying to a cyanogen bromide-derivatized Sepharose column coupled to murine OPGbp [158-316] and eluting with 0.1M glycine, pH 2.9. In experiments done under my direction, antisera from rabbits immunized with EF loop6-Cys peptide were purified by applying to a Sulfolink column (Pierce) coupled to EF loop-Cys6 peptide and eluting under conditions recommended by the manufacturer. The affinity purified anti-OPGbp antibodies are designated by the OPGbp peptide or polypeptide used for immunization.

8. In experiments done under my direction, the affinity purified anti-OPGbp antibodies were tested for binding

to murine OPGbp[158-316] or human OPGbp[159-317] by EIA generally as described on p. 48, line 28 to p. 50, line 5 in the '315 application and by the following procedure: Each well of a Costar EIA plate (Catalog no. 3590) was coated with 0.1 ml of either a 5 µg/ml human OPGbp[159-317] or a 5 µg/ml murine OPGbp[158-316] solution in Carbonate/Bicarbonate buffer / pH9.6 at 4°C overnight with agitation. After overnight coating, the solution was removed from the plates and 200 µl of 5% BSA blocker (Kirkegaard-Perry (K-P) Labs, Gaithersburg, MD, Catalog no. 50-61-00) at a 1:2 dilution was added to each well and the plates were incubated at room temperature for 1 hour. After incubation, the plates were washed two times with 1X K-P wash solution (Catalog no. 50-63-00). The affinity purified antibodies were serially diluted into PBS (lacking Ca2+ or Mg2+) containing 0.1% Tween-20 (PBS-Tween). 100 µl of each dilution was added to appropriate wells of plates coated with either human OPGbp[159-317] or murine OPGbp[158-316] and incubated for 40 minutes at room temperature with agitation. Following this incubation, the plates were washed three times with the 1X K-P wash solution. To each well of the plate was then added 100 µl of a 1:3000 dilution of Protein A-horseradish peroxidase conjugate (Boehringer Mannheim Biochemicals, Catalog no. 605-295) in PBS-Tween and the plates were incubated 40 minutes further at room temperature with agitation. The plates were then washed four times with the 1X K-P wash solution. Following this final wash, 100 µl of ABTS substrate (K-P Labs, Catalog no. 50-66-01) was added to each well and the absorbance at 405nm was determined at various times using a SPECTRAmax 340 plate reader (Molecular Devices). The results presented in Attachment No. 1 show that the affinity purified anti-OPGbp antibodies bound to both murine and human OPGbp regardless of the OPGbp peptide or polypeptide used for immunization.

9. The affinity purified anti-BB' loop-Cys, anti-EF loop6-Cys, and anti-human OPGbp[159-317] antibodies were provided

to the Analytical Resources Department at Amgen Inc. in order to test for inhibition of osteoclastogenesis *in vitro*. Based upon information provided to me, the mouse bone marrow assay generally as described in Example 8 of the specification was used, with the modification that 20 ng/ml of human OPGbp[159-317], rather than murine OPGbp[158-316], was included. The results provided to me are shown in Attachment No. 2 and indicate that antibodies raised to BB'loop-Cys peptide and human OPGbp[159-317] block osteoclastogenesis whereas antibodies raised to the EF loop6-Cys peptide do not appear to exhibit a significant effect. In the attachment, the A405 values for the anti-human OPGbp and anti-EF loop6-Cys antibodies were normalized to a similar maximum response in order to more readily compare the activities.

10. The affinity purified anti-human OPGbp[159-317] antibodies were provided to the Pathology Department at Amgen Inc. in order to test for effects on bone density. Based on information provided to me, the following *in vivo* assay was used. Male BDF1 mice aged three to four weeks were administered varying doses of affinity purified anti-human OPGbp antibody by daily subcutaneous injection in carrier (PBS/0.1% BSA) starting on day 1. The mice were then x-rayed on day 5. All mice in each treatment group (eight mice per group) and in the PBS/0.1% BSA control group were x-rayed on a single film. The proximal tibial metaphyseal region was compared between pairs of control and treated tibias and scored as a "+" if the treated tibia was denser by visual assessment than the control giving the eight scores shown below. An arbitrary score of 5/8 was required for a "positive" result. The results which were provided to me are shown in Attachment No. 3 and clearly indicate that the anti-human OPGbp increased bone density in this assay at all doses tested.

11. It is clear that the anti-OPGbp antibodies obtained using materials and procedures described in the '315 application block osteoclast formation *in vitro* and promote an increase in bone density *in vivo*. It is apparent that the

specification enables one skilled in the art without undue experimentation to obtain antibodies which are useful for practicing the methods claimed in the '315 application.

12. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 10 of the United States Code and that such willful false statements may jeopardize the validity of the instant patent application or patent issuing thereon.

Date: 8-18-2000

John K. Sullivan